Amendments to the Claims

Please withdraw Claims 49-53, 73 and 79, amend Claims 48, 54-56, 62, 73, 79, 93, 100, and add New Claims 110-117.

This listing of the Claims will replace all prior versions and listings of the claims in this patent application.

1. (Withdrawn) A vector system for transfection and recombinant polypeptide expression in a mammalian host cell comprising:

(a) a first cistron encoding a transactivator protein under control of a first promoter; and

(b) a second cistron encoding an apoptosis-protective protein under the control of the first promoter or optionally under the control of a second promoter;

wherein the first and the second cistron are contained in one or more vectors.

2. (Withdrawn) The vector system of Claim 1, further comprising a third cistron encoding at least one desired polypeptide under control of a third promoter, wherein said third promoter is responsive to the transactivator protein and wherein the first, the second, and the third cistrons are contained in one or more vectors.

3. (Withdrawn) The vector system of Claim 2, further comprising one or more additional cistrons each encoding a desired polypeptide under control of a promoter responsive to the transactivator protein.

4. (Withdrawn) The vector system of Claim 2, wherein said polypeptide is a single chain antibody or a heavy or light chain of an antibody or antibody fragment.

5. (Withdrawn) The vector system of Claim 1, wherein the first and second cistrons are on one vector and the first cistron lies downstream of the second cistron.

6. (Withdrawn) The vector system of Claim 1, wherein the first cistron encodes a CREB protein or a variant thereof.

7. (Withdrawn) The vector system of Claim 6, wherein the CREB protein variant is CREB variant Y134F.

8. (Withdrawn) The vector system of Claim 6, wherein the second cistron encodes an adenoviral E1b-19K protein, a Bcl-2 protein, or a Bcl-2 protein having a deletion in the regulatory loop domain.

9. (Withdrawn) The vector system of Claim 1, wherein the first cistron encodes an adenoviral E1a polypeptide or a variant thereof.

10. (Withdrawn) The vector system of Claim 9, wherein the adenoviral E1a variant comprises a mutation in CR1.

11. (Withdrawn) The vector system of Claim 10, wherein the adenoviral E1a variant comprises a 47H mutation.

12. (Withdrawn) The vector system of Claim 1, wherein the second cistron encodes an apoptosis protective protein selected from the group consisting of a dominant negative mutant of p53, a protein that interacts with BAX, a protein that interacts with BAK, an inhibitor of apoptosome formation, and a downstream apoptosis inhibitor.

13. (Withdrawn) The vector system of Claim 1, wherein the second cistron encodes an adenoviral E1b-19K protein, a Bcl-2 protein, or a Bcl-2 protein having a deletion in the regulatory loop domain.

14. (Withdrawn) The vector system of Claim 1, wherein the first or second promoter is an efficient heterologous promoter.

15. (Withdrawn) The vector system of Claim 1, wherein the first or second promoter is a RSV-LTR promoter, a SV -40 promoter, or a cytomegalovirus promoter.

16. (Withdrawn) The vector system of Claim 2, wherein the third promoter comprises a CREB-binding element or a 19bp repeat from a hCMV-MIE enhancer.

17. (Withdrawn) The vector system of Claim 2, wherein the third promoter comprises a TATAA transcription initiation signal.

18. (Withdrawn) The vector system of Claim 2, wherein the third promoter is a hCMV-MIE promoter having a TATAA box region.

19. (Withdrawn) The vector system of Claim 2, wherein the third cistron is associated with a ubiquitous chromatin opening element,. an insulator, or a barrier element.

20. (Withdrawn) The vector system of Claim 19, wherein the ubiquitous chromatin opening element comprises an extended methylation-free CpG-island.

21. (Withdrawn) The vector system of Claim 19, wherein the ubiquitous chromatin opening element comprises a hnRNP A2 promoter.

22. (Withdrawn) A method of expressing a desired recombinant polypeptide in a mammalian host cell comprising introducing to the mammalian host cell:

(a) a first cistron encoding a trans activator protein under control of a first promoter;

(b) a second cistron encoding an apoptosis-protective protein under control of the first promoter or optionally under control of a second promoter; and

(c) a third cistron encoding the desired polypeptide under control of a third promoter;

wherein said third promoter is responsive to the transactivator protein.

23. (Withdrawn) The method of Claim 22, wherein the third cistron is associated with a ubiquitous

chromatin opening element, an insulator, or a barrier element.

24. (Withdrawn) The method of Claim 22, wherein the host cell is selected from the group consisting of a CHO cell, a mouse myeloma cell, a mouse hybridoma cell, a rat myeloma cell, and a rat hybridoma cell.

25. (Withdrawn) The method of Claim 24, wherein the host cell is a cell capable of growing in a suspension.

26. (Withdrawn) The method of Claim 24, wherein the host cell is a YB2/0 rat hybridoma cell.

27. (Withdrawn) The method of Claim 22, wherein the first or second promoter is an efficient heterologous promoter.

28. (Withdrawn) The method of Claim 22, wherein the transactivator and the apoptotic protective protein are homologous to the endogenous transactivator and apoptotic protective proteins of the host cell.

29. (Withdrawn) The method of Claim 22, wherein the first cistron encodes a transactivator protein selected from the group consisting of an E1a protein, a CREB protein, and variants thereof.

30. (Withdrawn) The method of Claim 29, wherein the first cistron encodes CREB variant Y134F.

31. (Withdrawn) The method of Claim 22, wherein the first cistron encodes a CREB protein or a variant thereof, and the second cistron encodes a Bcl-2 protein or a Bcl-2 protein having a deletion in the regulatory loop domain.

32. (Withdrawn) The method of Claim 22, wherein the first cistron encodes a variant E1a protein with a mutation in CR1, and the second cistron encodes an E1b-19K protein, a Bcl-2 protein, or a Bcl-2 protein having a deletion in the regulatory loop domain.

33. (Withdrawn) The method of Claim 22, wherein the second cistron encodes an apoptosis-protective protein selected from the group consisting of a dominant negative mutant of p53, a protein that interacts with BAX, a protein that interacts with BAK, an inhibitor of apoptosome formation, and a downstream apoptosis inhibitor.

34. (Withdrawn) The method of Claim 22, wherein the second cistron encodes an adenovirus E1b-19K protein, a Bcl-2 protein, or a Bcl-2 protein having a deletion in the regulatory loop domain.

35. (Withdrawn) The method of Claim 22, wherein said polypeptide is a single-chain antibody or a heavy or light chain of an antibody or antibody fragment.

36. (Withdrawn) The method of Claim 22, wherein said polypeptide is a part of a library of polypeptides.

37. (Withdrawn) A mammalian host cell for recombinant polypeptide expression comprising a first cistron encoding a transactivator protein and a second cistron encoding an apoptosis-protective protein that prevents cell-killing due to expression of the transactivator protein.

38. (Withdrawn) The host cell of Claim 37, further comprising a third cistron encoding one or more desired polypeptide under the control of a promoter responsive to the transactivator protein.

39. (Withdrawn) The host cell of Claim 37, wherein the third cistron is associated with a ubiquitous chromatin opening element, an insulator, or a barrier element.

40. (Withdrawn) The host cell of Claim 37, wherein the transactivator protein is expressed from an efficient heterologous promoter.

41. (Withdrawn) The host cell of Claim 37, wherein the first cistron encodes a CREB protein or a variant thereof, and the second cistron encodes a Bcl-2 protein or a Bcl-2 protein having a deletion in the regulatory loop domain.

42. (Withdrawn) The host cell of Claim 37, wherein the first cistron encodes a Ela variant comprising a mutation in CR1.

43. (Withdrawn) The host cell of Claim 37, wherein said cell is a CHO cell or a YB2/0 cell.

44. (Withdrawn) The host cell of Claim 37, wherein said cell is a cell capable of growing in a suspension.

45. (Withdrawn) The host cell of Claim 37, wherein said host cell is from an established cell line.

46. (Withdrawn) The host cell of Claim 37, wherein said host cell is a non-human mammalian host cell.

47. (Withdrawn) A method for producing a recombinant protein comprising culturing the host cell of Claim 37 in a suitable medium such that the one or more desired proteins are secreted into the medium.

48. (Currently Amended) A vector system for transfection and recombinant polypeptide expression in a mammalian host cell comprising:

a first cistron encoding a transactivator protein under control of a first promoter; ~~and~~

a second cistron encoding an apoptosis-protective protein under control of the first promoter or optionally under control of a second promoter, wherein said second promoter is responsive to the transactivator protein;

a third cistron encoding a heavy chain of an antibody or antibody fragment under control of a third promoter, wherein said third promoter is responsive to the transactivator protein;

a fourth cistron encoding a light chain of an antibody or antibody fragment under control of a fourth promoter;

wherein the third cistron and the fourth cistron are contained in a retroviral vector, wherein the third cistron and fourth cistron are associated with a ubiquitous chromatin opening element (UCOE), an insulator, or a barrier element, and wherein the third cistron and the fourth cistron are separated by an internal ribosome entry site (IRES); and

wherein the first and the second cistrons are contained in one or more vectors.

49. (Withdrawn) The vector system of Claim 48, further comprising a third cistron encoding at least one desired polypeptide under control of a third promoter, wherein said third promoter is responsive to the transactivator protein and wherein the first, the second, and the third cistrons are contained in one or more vectors.

50. (Withdrawn) The vector system of Claim 49, further comprising one or more additional cistrons each encoding a desired polypeptide under control of a promoter responsive to the transactivator protein.

51. (Withdrawn) The vector system of Claim 49, wherein said polypeptide is a single chain antibody or a heavy or light chain of an antibody or antibody fragment.

52. (Withdrawn) The vector system of Claim 48, wherein the first and second cistrons are on one vector and the first cistron lies downstream of the second cistron.

53. (Withdrawn) The vector system of Claim 49, wherein the third cistron is associated with a ubiquitous chromatin opening element (UCOE), an insulator, or a barrier element.

54. (Currently Amended) The vector system of Claim ~~53~~ 48, wherein the ubiquitous chromatin opening element (UCOE) comprises an extended methylation-free CpG-island.

55. (Currently Amended) The vector system of Claim ~~53~~ 48, wherein the ubiquitous chromatin opening element (UCOE) comprises an hnRNP A2 promoter.

56. (Currently Amended) A method of expressing a desired recombinant polypeptide in a mammalian host cell comprising introducing to the mammalian host cell:

a first cistron encoding a transactivator protein under control of a first promoter, wherein the transactivator is expressed at a level that could cause host cell death without an apoptosis-protective protein;

a second cistron encoding ~~an~~ the apoptosis-protective protein under control of the first promoter or optionally under control of a second promoter, wherein the apoptosis-protective protein prevents death of the host cell from the transactivator; and

a third cistron encoding the desired polypeptide under control of a third promoter; wherein said third promoter is responsive to the transactivator protein.

57. (Previously Presented) The method of Claim 56, wherein the third cistron is associated with a ubiquitous chromatin opening element (UCOE), an insulator, or a barrier element.

58. (Previously Presented) The method of Claim 56, wherein the host cell is selected from the group consisting of a CHO cell, a mouse myeloma cell, a mouse hybridoma cell, a rat myeloma cell, and a rat hybridoma cell.

59. (Previously Presented) The method of Claim 56, wherein the transactivator and the apoptotic protective protein are homologous to the endogenous transactivator and apoptotic protective proteins of the host cell.

60. (Previously Presented) The method of Claim 56, wherein said polypeptide is a single-chain antibody or a heavy or light chain of an antibody or antibody fragment.

61. (Previously Presented) The method of Claim 56, wherein said polypeptide is a part of a library of polypeptides.

62. (Currently Amended) A mammalian host cell for recombinant polypeptide expression comprising a first cistron encoding a transactivator protein, a second cistron encoding an apoptosis-protective protein that prevents cell-killing due to the expression of the transactivator protein, and a third cistron encoding one or more desired polypeptides under control of a promoter responsive to the transactivator protein, wherein the transactivator is expressed at a level that could cause death of the host cell in the absence of the apoptosis-protective protein, and wherein the apoptosis-protective protein prevents cell death from the transactivator.

63. (Previously Presented) The host cell of Claim 62, wherein said cell is a CHO cell or a YB2/0 cell.

64. (Previously Presented) A method for producing a recombinant protein comprising culturing the host cell of Claim 62 in a suitable medium such that the one or more desired proteins are secreted into the medium.

65. (Withdrawn) A nucleic acid comprising:

a cistron encoding a desired antibody polypeptide under control of a promoter;

a ubiquitous chromatin opening element (UCOE) operably linked to the cistron; and

a retroviral vector, wherein the cistron, the promoter, and the UCOE are contained in the retroviral vector.

66. (Withdrawn) The nucleic acid of Claim 65, wherein the promoter is responsive to a transactivator protein capable of enhancing expression of the polypeptide.

67. (Withdrawn) The nucleic acid of Claim 65, wherein the antibody polypeptide is a single chain antibody or a heavy or light chain of an antibody or antibody fragment.

68. (Withdrawn) The nucleic acid of Claim 65, further comprising a second cistron encoding a second desired antibody polypeptide under control of the promoter, wherein the second cistron is contained in the retroviral vector, and the first and second cistrons are separated by an internal ribosome entry site (IRES).

69. (Withdrawn) The nucleic acid of Claim 68, wherein the first cistron encodes an antibody light chain or an antibody light chain fragment, and the second cistron encodes an antibody heavy chain or an antibody heavy chain fragment.

70. (Withdrawn) The nucleic acid of Claim 65, wherein the UCOE comprises an extended methylation-free CpG-island.

71. (Withdrawn) The nucleic acid of Claim 65, wherein the UCOE comprises an hnRNP A2 promoter.

72. (Withdrawn) A vector system comprising a plurality of the nucleic acids of Claim 65, wherein a first nucleic acid comprises a first cistron encoding an antibody light chain or an antibody light chain fragment, and a second nucleic acid comprises a second cistron encoding an antibody heavy chain or an antibody heavy chain fragment.

73. (Withdrawn) A vector system comprising the nucleic acid of Claim 65, further comprising-a cistron encoding a transactivator protein under control of a second promoter, said transactivator protein capable of enhancing expression of the antibody polypeptide, and

74. (Withdrawn) The vector system of Claim 73, further comprising a cistron encoding an apoptosis-protective protein under control of the second promoter or optionally under control of a third promoter.

75. (Previously Presented) The vector system of Claim 50, wherein two of the cistrons encoding the desired polypeptides are on one vector and are separated by an internal ribosome entry site (IRES).

76. (Previously Presented) The vector system of Claim 75, further comprising a ubiquitous opening element (UCOE) operably linked to the two cistrons encoding the polypeptides.

77. (Previously Presented) The vector system of Claim 76, wherein the cistrons encoding the polypeptides are contained in a retroviral vector.

78. (Withdrawn) A vector system comprising:

a first cistron encoding a first desired antibody polypeptide under control of a first promoter;

a second cistron encoding a second desired antibody polypeptide under control of the first promoter;

a third cistron encoding a transactivator protein under control of a second promoter;

wherein the first and second cistrons are on a retroviral vector and are separated by an internal ribosome entry site (IRES), and the third cistron is on the same retroviral vector or optionally on a different vector.

79. (Withdrawn) The vector system of Claim 78, further comprising a cistron encoding an apoptosis-protective protein under control of the second promoter or optionally under control of a third promoter.

80. (Withdrawn) A method of expressing a desired antibody in a mammalian host cell comprising the steps of:

growing the mammalian host cell containing a nucleic acid, said nucleic acid comprising:

a cistron encoding an antibody polypeptide under control of a promoter;

a ubiquitous chromatin opening element (UCOE) operably linked to the cistron; and

a retroviral vector, wherein the cistron, the promoter, and the UCOE are contained in the retroviral vector; and

expressing the antibody polypeptide in the mammalian host cell.

81. (Withdrawn) The method of Claim 80, wherein the antibody polypeptide is a single chain antibody or a heavy or light chain of an antibody or antibody fragment.

82. (Withdrawn) The method of Claim 80, further comprising introducing to the host cell one or more of the nucleic acids.

83. (Withdrawn) The method of Claim 82, wherein a first nucleic acid comprises a cistron encoding an antibody light chain or an antibody light chain fragment, a second nucleic acid comprises a cistron encoding an antibody heavy chain or an antibody heavy chain fragment.

84. (Withdrawn) The method of Claim 80, wherein the nucleic acid or nucleic acids are introduced to the host cell by infection.

85. (Withdrawn) The method of Claim 80, wherein the nucleic acid further comprises a second cistron encoding a second desired antibody polypeptide wherein the second cistron is contained in the retroviral vector, and the first and second cistrons are separated by an internal ribosome entry site (IRES).

86. (Withdrawn) The method of Claim 85, wherein the first cistron encodes an antibody light chain or an antibody light chain fragment, the second cistron encodes an antibody heavy chain or an antibody heavy chain fragment.

87. (Withdrawn) The method of Claim 80, wherein the host cell is selected from the group consisting of a CHO cell, a mouse myeloma cell, a mouse hybridoma cell, a rat myeloma cell, and a rat hybridoma cell.

88. (Withdrawn) The method of Claim 56, further comprising introducing to the mammalian host cell one or more additional cistrons each encoding a desired polypeptide under control of a promoter responsive to the transactivator protein.

89. (Withdrawn) The method of Claim 88, wherein two of the cistrons encoding the desired polypeptides are on one vector and are separated by an internal ribosome entry site (IRES).

90. (Withdrawn) The method of Claim 89, further comprising introducing to the mammalian host cell a ubiquitous opening element (UCOE) operably linked to the two cistrons encoding the polypeptides.

91. (Withdrawn) The method of Claim 90, wherein the two cistrons encoding the polypeptides are contained in a retroviral vector.

92. (Withdrawn) A method of expressing a desired antibody in a mammalian host cell comprising the steps of:

growing the mammalian host cell containing a nucleic acid, said nucleic acid comprising:

a first cistron encoding a first desired antibody polypeptide under control of a first promoter;

a second cistron encoding a second desired antibody polypeptide under control of the first promoter;

a third cistron encoding a transactivator protein under control of a second promoter;

wherein the first and second cistrons are on a retroviral vector and are separated by an internal ribosome entry site (IRES), and the third cistron is on the same retroviral vector or optionally on a different vector; and

expressing the antibody polypeptide in the mammalian host cell.

93. (Currently Amended) ~~The method of Claim 92,~~ A method of expressing a desired antibody in a mammalian host cell comprising the steps of:

growing the mammalian host cell containing a nucleic acid, said nucleic acid comprising:

a first cistron encoding a first desired antibody polypeptide under control of a first promoter;

a second cistron encoding a second desired antibody polypeptide under control of the first promoter;

a third cistron encoding a transactivator protein under control of a second promoter;

~~wherein said nucleic acid further comprises~~ a fourth cistron encoding an apoptosis-protective protein under control of the second promoter or optionally under control of a third promoter.

wherein the first and second cistrons are on a retroviral vector and are separated by an internal ribosome entry site (IRES), the third cistron is on the same retroviral vector or optionally on a different vector, and the fourth cistron is on the same retroviral vector or optionally on a different vector;

expressing the transactivator proteins at level that can cause host cell death in the absence of the apoptosis-protective protein;

expressing the apoptosis protective protein at a level to inhibit death of the host cell from the transactivator; and

expressing the antibody polypeptide in the mammalian host cell.

94. (Withdrawn) A mammalian host cell comprising a nucleic acid, said nucleic acid comprising:

a cistron encoding an antibody polypeptide under control of a promoter;

a ubiquitous chromatin opening element (UCOE) operably linked to the cistron; and

a retroviral vector, wherein the cistron, the promoter, and the UCOE are contained in the retroviral vector.

95. (Withdrawn) The host cell of Claim 94, wherein the antibody polypeptide is a single chain antibody or a heavy or light chain of an antibody or antibody fragment.

96. (Withdrawn) The host cell of Claim 94, further comprising one or more of the nucleic acids.

97. (Withdrawn) The host cell of Claim 94, wherein the nucleic acid further comprises a second cistron encoding a second desired antibody polypeptide wherein the second cistron is contained in the retroviral vector, and the first and second cistrons are separated by an internal ribosome entry site (IRES).

98. (Withdrawn) The host cell of Claim 97, wherein the first cistron encodes an antibody light chain or an antibody light chain fragment, and the second cistron encodes an antibody heavy chain or an antibody heavy chain fragment.

99. (Withdrawn) The host cell of Claim 94, wherein the host cell further comprises a cistron encoding a transactivator protein under control of a promoter.

100. (Currently Amended) A mammalian host cell comprising:

a retroviral vector, comprising a cistron encoding an antibody polypeptide under control of a first promoter and a ubiquitous chromatin opening element (UCOE) operably linked to the cistron; and

a cistron encoding a transactivator protein under control of a second promoter, wherein the transactivator protein is present in the host cell in amounts that could cause death of the host cell without an apoptosis protective protein;

~~The host cell of Claim 99, wherein the host cell further comprises~~ a cistron encoding the apoptosis-protective protein under control of a third promoter, wherein the apoptosis-protective

protein is present in the host cell in amounts sufficient to inhibit death of the host cell from the transactivator.

101. (Withdrawn) The host cell of Claim 94, wherein the UCOE comprises an extended methylation-free CpG-island.

102. (Withdrawn) The host cell of Claim 94 wherein the host cell is a CHO cell or a YB2/0 cell.

103. (Previously Presented) The host cell of Claim 62, further comprising one or more additional cistrons each encoding a desired polypeptide under control of a promoter responsive to the transactivator protein.

104. (Previously Presented) The host cell of Claim 103, wherein two of the cistrons encoding the desired polypeptides are on one vector and are separated by an internal ribosome entry site (IRES).

105. (Previously Presented) The host cell of Claim 104, further comprising introducing to the mammalian host cell a ubiquitous opening element (UCOE) operably linked to the two cistrons encoding the polypeptides.

106. (Previously Presented) The host cell of Claim 105, wherein the two cistrons encoding the polypeptides are contained in a retroviral vector.

107. (Withdrawn) A mammalian host cell comprising a nucleic acid, said nucleic acid comprising:

a first cistron encoding a first desired antibody polypeptide under control of a first promoter;

a second cistron encoding a second desired antibody polypeptide under control of the first promoter;

a third cistron encoding a transactivator protein under control of a second promoter;

wherein the first and second cistrons are on a retroviral vector and are separated by an internal ribosome entry site (IRES), and the third cistron is on the same retroviral vector or optionally on a different vector.

108. (Withdrawn) The mammalian host cell of Claim 107, wherein said nucleic acid further comprises a cistron encoding a transactivator-protective protein under control of the second promoter or optionally under control of a third promoter.

109. (Withdrawn) A method of producing an antibody polypeptide comprising culturing the host cell of Claim 94 in a suitable medium such that one or more desired antibody polypeptides are secreted into the medium.

110. (New) The method of claim 56, wherein the production rate of the desired polypeptide is enhanced at least two-fold by the combination of the transactivator and the apoptosis-protective protein.

111. (New) The method of claim 56, wherein the production rate of the desired polypeptide is enhanced at least five-fold by the combination of the transactivator and the apoptosis-protective protein.

112. (New) The mammalian host cell of claim 62, wherein the production rate of the desired polypeptide in the host cell is enhanced at least two-fold by the combination of the transactivator and the apoptosis-protective protein.

113. (New) The mammalian host cell of claim 62, wherein the production rate of the desired polypeptide in the host cell is enhanced at least five-fold by the combination of the transactivator and the apoptosis-protective protein.

114. (New) The method of claim 93, wherein the production rate of the desired polypeptide is enhanced at least two-fold by the combination of the transactivator and the apoptosis-protective protein.

115. (New) The method of claim 93, wherein the production rate of the desired polypeptide is enhanced at least five-fold by the combination of the transactivator and the apoptosis-protective protein.

116. (New) The mammalian host cell of claim 100, wherein the production rate of the desired polypeptide in the host cell is enhanced at least two-fold by the combination of the transactivator and the apoptosis-protective protein.

117. (New) The mammalian host cell of claim 100, wherein the production rate of the desired polypeptide in the host cell is enhanced at least five-fold by the combination of the transactivator and the apoptosis-protective protein.